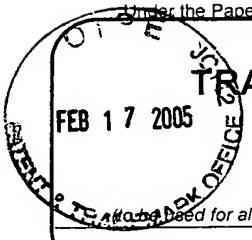
02/18/05

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.



TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	44

Application Number	10/010,114
Filing Date	November 13, 2001
First Named Inventor	Raymond H. Boutin
Art Unit	1632
Examiner Name	D. Crouch
Attorney Docket Number	AHP1CUSA

ENCLOSURES (Check all that apply)

- [✓] Fee Transmittal Form
- [✓] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [✓] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [ ] Other Enclosure(s) (please Identify below):

Remarks

Appeal Brief

Express Mail No. ER635173759US

Customer No. 38199

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Howson and Howson		
Signature	Cathy A. Kodroff		
Printed name	Cathy A. Kodroff		
Date	2-17-2005	Reg. No.	33,980

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name		Date	

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/17 (12-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL For FY 2005

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($)	500.00

Complete if Known	
Application Number	10/010,114
Filing Date	November 13, 2001
First Named Inventor	Raymond H. Boutin
Examiner Name	D. Crouch
Art Unit	1632
Attorney Docket No.	AHP1CUSA

METHOD OF PAYMENT (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 08-3040 Deposit Account Name: Howson and Howson

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES Fee ($)	FILING FEES Small Entity Fee ($)	SEARCH FEES Fee ($)	SEARCH FEES Small Entity Fee ($)	EXAMINATION FEES Fee ($)	EXAMINATION FEES Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 20 or HP =	_____	x _____	= _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 3 or HP =	_____	x _____	= _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims Fee ($)	Multiple Dependent Claims Fee Paid ($)
_____	_____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
_____ - 100 =	_____ / 50 =	_____ (round up to a whole number)	x _____	= _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): Appeal Brief	$500.00

SUBMITTED BY

Signature	Cathy A. Kodroff	Registration No. (Attorney/Agent) 33,980	Telephone 215-540-9200
Name (Print/Type)	Cathy A. Kodroff		Date February 17, 2005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	:	10/010,114	Confirmation No. 5743
Applicant	:	Raymond H. Boutin	
Filed	:	November 13, 2001	
TC/A.U.	:	1632	
Examiner	:	D. Crouch	
Docket No.	:	AHP1CUSA	
Customer No.	:	38199	

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22323-1450

BRIEF ON APPEAL

Sir:

This Appeal Brief is timely filed. A Notice of Appeal was filed by express mail on December 21, 2004. The appeal is from the Office Action dated September 22, 2004 and made final, which rejected pending claims 1, 2, 5-9 and 17-52.

The fee of $500.00 for filing this Appeal Brief is attached hereto. The Director is hereby authorized to charge any deficiency in any fees due with the filing of this paper, or credit any overpayment, to our Deposit Account, No. 08-3040.

I. Real party in interest

The real party in interest is the Applicant's assignee, Wyeth, a Delaware corporation located at Five Giralda Farms, Madison, New Jersey 07940.

II. Related appeals and interferences

None.

Express Mail No. ER635173759US

III. Status of claims

The pending claims are claims 1-9 and 17-52. Claims 1-2, 5-9 and 17-52 stand rejected. Claim 3 is withdrawn. Claim 4 is objected to, but indicated to be allowable if rewritten in conformity with the election. Claims 10-16 have been canceled. Claim 49 has replaced claim 3 as the elected claim for examination. Claims 1-2, 5-9, 17-48 and 50-52 are linking claims.

Claims 1-2, 5-9 and 17-52 are the subject of this appeal.

IV. Status of amendments

There are no outstanding amendments.

V. Summary of claimed subject matter

The invention as presented in the claims is a method for transferring a nucleic acid to cells by introducing a multifunctional molecular complex into cells. The novel multifunctional molecular complex[1] is composed of a nucleic acid composition and a novel transfer moiety[2]. The transfer moiety is composed of one or more cationic polyamine components non-covalently bonded to the nucleic acid, and one or more endosome membrane disruption promoting components.

The present invention provides a construct which is capable of targeting to a variety of host cells, penetrating the cell membrane and, in some embodiments, disrupting endosomes. Thus, the method of the invention provides a novel and nonobvious method of delivering nucleic acid molecules carried on the multifunctional molecular complex to cells.

---

1 The multifunctional molecular complex is the subject of the claims of issued grandparent and parent applications, now US Patent Nos. 6,127,170 and 6,379,965.

2 The transfer moiety is the subject of the claims of issued parent application, US Patent No. 6,379,965.

Thus, the method of the invention provides advantages over prior art methods of delivering molecules to cells, including viral and nonviral delivery vectors. Notably, novelty and nonobviousness of the method of the invention is not in dispute.

VI. Grounds of rejection to be reviewed on appeal

The issue on appeal is whether the examiner's rejection of claims 1-3, 5-9 and 17-52 under the provisions of 35 U.S.C. 112, first paragraph, should be reversed.

VII. Argument

The examiner has incorrectly rejected the claims under 35 USC 112, first paragraph. Applicant requests reversal of the outstanding rejection for the reasons set forth herein.

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms *as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same*, and shall set forth the best mode contemplated by the inventor of carrying out his invention. (emphasis added) 35. U.S.C. 112, first paragraph.

> The test for enablement is whether "the experimentation needed to practice the invention [is] undue or unreasonable". (*Mineral Separation v. Hyde*, 242 U.S. 261, 270 (1916); i*n re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cit. 1988)). The factors to be considered include, but are not limited to: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill; the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and the quantity of experimentation needed to make or use the invention based on the content of the disclosure. (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)

A. The examiner is improperly requiring that the application demonstrate that a protein encoded by the nucleic acid molecule delivered by a multifunctional molecular complex according to the method of the invention confers a specific therapeutic benefit or another specific physiologic effect.

> The examiner has the initial burden to establish a reasonable basis to question enablement provided for the claimed invention. In re Wright, 999 F.2d. 1557, 1562 (Fed. Cir. 1993); MPEP 2164.04. . . . A specification disclosure which contains a teaching of the manner and process of making and using an invention *in terms which correspond to the scope to those used in describing and defining the subject matter sought to be patented* must be taken as being in compliance with the enablement requirement of 35 USC 112, first paragraph, unless there is no reason to doubt the objective truth of the statements contained therein which must be relied upon for enabling support. MPEP 2164.04

The examiner has failed to establish a reasonable basis to question the enablement of claims which recite a method for the transfer of a nucleic acid composition to cells, comprising the step of introducing a multifunctional molecular complex into cells with respect to cells *in vivo*. The claims do not recite the term "in vivo".

Applicant needs only establish that the application enable one of skill in the art to make and use a method for transfer nucleic acid compositions to cells by introducing multifunctional molecular complex of the invention which comprises, inter alia, a nucleic acid composition, without undue experimentation. Since the claims do not require a therapeutic effect, Applicant need not demonstrate such an effect in order to enable the claimed subject matter.

> The examiner has argued that the claimed methods are not enabled for methods of transfer where the target cells are contained in a body or *in vivo*. The examiner further argues that because applicant has broadly disclosed treatment of any disease, but stated to specific disease, the examiner believes it appropriate to rely upon the general teachings in the art of gene therapy and cancer gene therapy at the time of filing, *i.e.*, September 28, 1994[3]. In so doing, the examiner has cited a number of publications describing the problems of delivery via plasmid and viral

[3] Page 3, Office Action dated 9/22/04.

> vectors in order to support the unpredictability of the art[4]. The examiner argues that these articles establish that the art reported problems relating to cellular delivery and expression, and nucleic acid degradation *in vivo*. as it relates to plasmid and/or viral-based delivery vehicles.

Applicant disagrees. The articles cited by the examiner describe problems associated with viral-based delivery vehicles and plasmid-based delivery vehicles.

Viral-based and plasmid delivery vehicles are not pertinent to the field of the present invention, as the present invention does not rely upon transfection by a plasmid or infection by a viral-based delivery vehicle. In fact, the examiner recognizes that the applicant's invention is directed to use of non-viral means, in essence, dismissing all of the concerns in these documents relating to delivery via plasmid and viral vectors she previously relied upon in this same action[5].

> The examiner further relies upon Treco et al, Non-Viral Gene Therapy, Molecular Medicine Today, Vol. 1: 314-321 (1995)[6] for statements made therein relating to the disadvantages of receptor mediated update of DNA.

However, the non-viral delivery system described by the Treco article as receptor mediated gene transfer involves "the injection of a DNA-protein complex into the animal, where a specific cell-surface receptor binds the protein portion of the complex, resulting in complex update by endocytosis". This differs from multifunctional molecular complex of the present invention. Further, the examiner apparently has disregarded the discussion of the DNA-lipid complexes, which are non-viral delivery systems which the authors report have a number of advantages. The examiner has not provided any reasoning as to why these compositions of the invention would be expected to have the same problems associated with the DNA-protein complexes described by Treco while disregarding the positive discussion by the authors of the DNA-lipid complex delivery systems. In fact,

---

4 Pages 3-5, Office Action dated 12/8/2003.

5 Page 5, Office Action dated 12/8/2003.

6 It is noted that this publication post-dated the filing date of the present application.

the present invention is more analogous to DNA-lipid complex delivery systems than the DNA-protein complex described by the Treco article.

> The examiner has argued that the multifunctional molecular complex would have to deliver the complexed nucleic acid to the nucleus of a target cell in order for transcription to occur, and that there can be no protein production without first mRNA production[7].

This basis for the rejection must fail. The examiner is reading language into the claims which does not exist. As previously summarized, the method invention provides a method for transferring a nucleic acid sequence into a cell, regardless of any *specific* physiologic effect of the encoded protein.

The examiner has admitted that the specification is "enabling for methods for the transfer of a nucleic acid composition to cells in culture comprising introducing a multifunctional molecular complex to cells where the complex comprises a nucleic acid encoding a therapeutic protein or polypeptide and a transfer moiety"[8]. However, there is no scientific support for the examiner's position that transcription and translation of a protein would be different in cell culture than *in vivo* once the cell membrane has been penetrated and the nucleic acid delivered by the vehicle of the present invention.

It is well-established law that in challenging enablement, which is otherwise to be presumed adequate on the face of the document:

> The factors to be considered include, but are not limited to: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill; the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and the quantity of experimentation needed to make or use the invention based on the content of the disclosure. (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)

---

7 Page 3, Office Action dated 9/22/2004.

8 Page 2, Office Action dated 9/22/2004.

Applicant submits that this examiner has failed improperly applied this standard by reading the claims to require a specific therapeutic result. This language is not recited in the claims. For the reasons set forth above, Applicant submits that the invention is enabled.

B. If evidence beyond what is provided in the specification is required to establish enablement, the examiner has improperly disregarded the Declaration pursuant to 37 CFR §1.132 as evidence of the utility of the claimed method in vivo.

The examiner argues that for the method of the invention to be enabled, the multifunctional molecular complex would have to deliver the complexed nucleic acid to the nucleus of a target cell in order for transcription to occur, and that there can be no protein production without first mRNA production[9].

Without regard to the scientific accuracy of the examiner's statement, Applicant has nevertheless supplied additional evidence that a nucleic acid introduced into a cell *in vivo* transfers the nucleic acid and permits expression thereof in the cell. Notably, it is admitted by the examiner that the Declaration submitted by Applicant, demonstrates that a humoral and CTL response was raised following delivery of a nucleic acid encoding HSV gD by a multifunctional molecular complex.[10].

As stated in the Declaration[11], the immune response was generated against the encoded gD protein (and not against the multifunctional molecular complex). Paragraphs 11-15 of the Declaration demonstrated *in vivo* expression of the nucleic acid encoding the gD protein of herpes simplex virus type 2 (HSV-2) when transferred to the cell by the multifunctional molecular complex, according to the present invention. More particularly, the multifunctional molecular complex as described in the specification was

---

9 Page 3, Office Action dated 9/22/2004.

10 Page 4, Office Action dated 9/22/2004.

11 The Declaration, signed November 24, 1998, was submitted along with Applicant's March 5, 2004 response to the Office Action dated December 8, 2003.

prepared and administered to mice intramuscularly.[12] The expression of gD was measured via cellular and humoral responses against controls, revealing that the method of the invention generated increased gD expression over naked DNA or DNA complexed with bupivacaine.[13] The results, as described with supporting data by the declarant, indicate that DNA transfer is successful and that the DNA expression yields an immune response.[14]

Thus, the invention not only delivered the nucleic acid sequence encoding a gD protein, but also expressed the protein in an appropriate manner in the cell. This establishes that *in vivo* delivery of a nucleic acid sequence by a multifunctional molecular complex of the invention was achieved.

The examiner argues that the evidence in the declaration is not persuasive because the encoded protein is an immunogen and not a therapeutic protein. However, the examiner provides no basis in science for the basis that the cell is capable of distinguishing between the nature of encoded proteins prior to their expression, *i.e.*, there is no scientific basis for distinguishing between delivery of encoded therapeutic proteins vs. encoded vaccinal proteins.

Thus, the Declaration should be accepted as evidence that method of the invention is useful for transferring a nucleic acid composition into cells *in vivo* and that these cells express the protein encoded by that nucleic acid composition.

Further, the Declaration established the ability of the multifunctional molecular complex to deliver a nucleic acid composition to a variety of cell types. The examiner has provided no basis for the position that expression in vivo is not predictable from expression in vivo, once transfer of the nucleic acid composition has been established.

---

[12] Declaration, paragraph 11.

[13] Declaration, paragraphs 12-14.

[14] Declaration, paragraph 15.

As indicated in the Declaration, *in vivo* delivery of a nucleic acid according to the invention was verified by lympho-proliferation and standard ELISA assay methods to detect protein expression as compared to delivery of naked DNA and DNA complexed with bupivacaine.[15] Further, the Declaration indicates that the *in vivo* data correlates with *in vitro* data from other cell types presented in the Declaration, which "demonstrates the ability of the multifunctional molecular complexes of the invention to mediate transfection of a variety of cell types."[16] The other cell types evaluated were human rhabdomyosarcoma (RD), human hepatocellular carcinoma (HUH 7), human hepatocytes (FOCUS), human hepatoblastoma (HEP 2), mouse embryo (NIH/3T2), human colon carcinoma (LS 180), SV40 transformed monkey kidney (COS-1), human osteosarcoma (U-2 OS), adenovirus-transformed human kidney cells (293), human breast cancer (MCF7), human neuronal blastoma (SY5Y), and primary human leukocytes.[17]

Additionally, four exemplary multifunctional molecular complexes as described in the specification were prepared and tested, using transfer moieties selected from bis-octyl-bis-guanido spermine, cholesteryl spermidine, benzyldodecyl spermidine, and cholesterol spermine.[18] The results obtained from each of the four exemplary complexes showed improved transfection over control (DNA alone).[19]

Thus, Applicant has demonstrated that one of ordinary skill in the art at the time of filing would be able to practice the invention without undue experimentation.

---

15 Declaration at paragraphs 11-14.

16 Declaration at paragraph 15.

17 Declaration at paragraph 9.

18 Declaration at paragraph 6.

19 Declaration at paragraph 7 and Exhibits 1 through 4 to the Declaration.

Reversal of the examiner's rejection of the claims under appeal (claims 1-2, 5-9 and 17-52) is requested.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for Applicants

By 

Cathy A. Kodroff
Registration No. 33,980
Spring House Corp. Center
Box 457
Spring House, PA 19477
(215) 540-9200

Claims Appendix

Claim 1(Original): A method for the transfer of a nucleic acid composition to cells, comprising the step of introducing a multifunctional molecular complex into cells,

wherein said multifunctional molecular complex comprises:

A) a nucleic acid composition; and

B) a transfer moiety comprising

(i) one or more cationic polyamine components, wherein each cationic polyamine is non-covalently bound to said nucleic acid composition and comprises from three to twelve nitrogen atoms; and

(ii) one or more endosome membrane disruption promoting components attached to at least one nitrogen atom of at least one of said polyamine components through an alkyl, carboxamide, carbamate, thiocarbamate, or carbamoyl bridging group, said one or more endosome membrane disruption promoting components independently selected from (a) at least one lipophilic long chain alkyl group or (b) a fusogenic peptide, cholic acid or cholesteryl group or a derivative thereof;

wherein said multifunctional molecular complex transfers said nucleic acid composition to said cells.

Claim 2(Original): A method according to Claim 1 wherein said nucleic acid composition is a nucleic acid molecule that comprises a nucleotide sequence that encodes a peptide or protein, or serves as a template for a nucleic acid molecule.

Claim 3(Withdrawn): A method according to Claim 2 wherein the peptide, protein or nucleic acid molecule is selected from the group consisting of vaccines; foodstuffs and nutritional supplements; compounds of agricultural significance; herbicides and plant growth regulants; insecticides; miticides; rodenticides; and fungicides; compounds useful in animal health; parasiticides; nematocides.

Claim 4(Original): A method according to Claim 1 wherein the target cells are cultures of host cells comprising microorganism cells of bacteria, yeast, plant or mammalian cells; said cell cultures being maintained in accordance with fermentation techniques which maximize production of the peptide, protein or functional nucleic acid molecule being produced.

Claim 5(Original): A method according to Claim 1 wherein the nucleic acid composition comprises a nucleotide sequence that encodes a protein and is operably linked to regulatory sequences.

Claim 6(Original): A method according to Claim 1 wherein the nucleic acid composition comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of an antigen against which an immune response is desired, said nucleotide sequence being operably linked to regulatory sequences.

Claim 7(Original): The method according to claim 1, wherein the transfer moiety of said multifunctional molecular complex further comprises at least one receptor specific binding component which is a ligand for a receptor on a target cell.

Claim 8(Original): The method according to claim 1, wherein the cationic polyamine comprises the formula (1):

$$NR(R^3)\text{-}[\text{-}(CR^1R^2)_m\text{-}N(R^3)\text{-}]_n\text{-}(CR^1R^2)_m\text{-}NR(R^3)$$

(1)

wherein:

R, R^1 and R^2 are each independently selected from the group consisting of hydrogen and $C_{1\text{-}6}$ alkyl;

m in each occurrence is independently selected from the integers 2 through 5 inclusive;

n is selected from the integers 1 through 10 inclusive; and

R^3 is independently selected from the group consisting of hydrogen; $C_{1\text{-}6}$ alkyl, an endosome membrane disruption promoting component, and a receptor specific binding component, or $NR(R^3)$ is guanidino,

wherein said transfer moiety comprises at least one endosome membrane disruption promoting component attached to at least one nitrogen atom of at least one of said cationic polyamine components.

Claim 9(Original): The method according to claim 1, wherein the nucleic acid composition is a plasmid.

Claims 10-16. Currently Cancelled.

Claim 17(Original): The method according to claim 7, wherein the receptor specific binding component is attached through a bridging group to either (i) to a further nitrogen atom of at least one of said cationic polyamine components to which said one or more endosome membrane disruption promoting components is attached, or (ii) to a nitrogen atom of at least one further polyamine component which does not have attached thereto any endosome membrane disruption promoting component.

Claim 18(Original): The method according to claim 17, wherein the bridging group through which the receptor specific binding component is attached is selected from the group consisting of an alkyl, carboxamide, carbamate, thiocarbamate, and carbamoyl bridging group.

Claim 19(Original): The method according to claim 8, wherein said one or more endosome membrane disruption promoting components are independently selected from the group consisting of:

(a) $-B-(CR^1R^2)_j-C(R)_3$, where R is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, or $C(R)_3$ is C_6H_5 aromatic or absent; R^1 and R^2 are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl; j is an integer from 0 to 24 inclusive; and B is optionally absent, or is a bridging group of the formula:

(i) $-(CR^1R^2)_k-C(=O)-Z-$;

(ii) $-(CR^1R^2)_k-N(R)-C(=O)-Z-$;

(iii) $-(CR^1R^2)_k-N(R)-\{-C(=O)-CH_2-O-[-(CH_2)_2-O-]_l-(CH_2)_k-N(R)\}_p-C(=O)-Z-$; or

(iv) $-(CR^1R^2)_k-C(=O)-\{-N(R)-[-(CH_2)_2-O-]_l-CH_2-C(=O)\}_p-Z-$;

where k is, independently, an integer from 1 to 11 inclusive, l is an integer from 0 to 30 inclusive, and p is an integer from 1 to 3 inclusive; R is independently defined as above or is absent, R^1 and R^2 are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl; and Z is O, OH, S, N(R), or is absent;

(b) $-B-(R^4)R$, where R, R^1 and R^2 are each independently defined as above; B cannot be absent and is a bridging group independently selected from groups (i) through (iv) above, and additionally from the group of the formula:

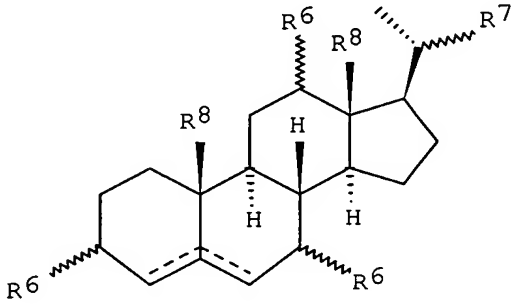
(v) $-(CR^1R^2)_{j=}-X-$, where j= is an integer from 1 to 8 inclusive; R^1 and R^2 are each independently defined as above;

X is O, S, N(R), or absent; and

R^4 is independently selected from the group consisting of:

(i) fusogenic peptides comprising spike glycoproteins of enveloped animal viruses;

(ii) cholic acid derivatives of the formula (2):



(2)

where:

~~www~~ represents a bond of unspecified stereochemistry;

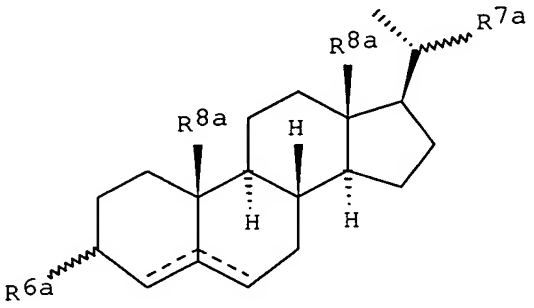
--- represents a single or double bond, forming a saturated or unsaturated portion of the ring system, provided that they cannot both be unsaturated at the same time, whereby the ring system must be either Δ4 or Δ5;

R^6 is -H, -OH, $-CO_2H$, $-C(=O)NH_2$, $-OC(=O)NH_2$, $-NH_2$, or $-O(CH_2CH_2O)_{n=}H$, where n= is an integer from 1 to 6 inclusive;

R^7 is a radical that forms the point of attachment of the cholic acid derivative, comprising $-C_{1-6}$ alkyl- or $-C_{1-6}$ alkylcarbonyl-; and

R^8 is C_{1-6} alkyl; and

(iii) cholesteryl derivatives of the formula (3):



(3)

where:

~~www~~ represents a bond of unspecified stereochemistry;

--- represents a single or double bond, forming a saturated or unsaturated portion of the ring system, provided that they cannot both be unsaturated at the same time, whereby the ring system must be either Δ4 or Δ5;

R^{6a} is a radical that forms the point of attachment of the cholesteryl derivative, comprising -C_{1-6} alkyl-, -OC(=O)-, or -OCH_2C(=O)-;

R^{7a} is C_{1-6} alkyl; and

R^{8a} is C_{1-6} alkyl.

Claim 20(Original): The method according to claim 8, wherein R^3 has the formula:

-B-(R^5)-R, where B cannot be absent and is a bridging group independently selected from groups (i) through (v) inclusive; R is independently as defined or absent; and R^5 is a receptor specific binding component independently selected from the group consisting of:

(i) D-biotin;

(ii) β-3'-propionyl galactosyl-β1-4- thioglucoside;

(iii) N^2, N^6-bis(β-3'-propionyl galactosyl-β1-4-thioglucoside)lysine;

(iv) N^2, N^6-bis(β-3'-propionyl galactosyl-β1-4-thioglucoside)lysyl-N^6-(β-3'-propionyl galactosyl-β1-4-thioglucoside)lysine;

(v) 5-methyltetrahydrofolate;

(vi) folic acid;

(vii) folinic acid;

(viii) α-3'-propionyl thiomannoside;

(ix) α-3'-propionyl thiomannoside-6-phosphate; and

(x) an antibody which binds specifically to a cell membrane protein.

Claim 21(Original): The method according to claim 8, wherein the cationic polyamine has the formula: NH_2-$(CH_2)_3$-$N(R^3)$-$(CH_2)_4$-NH_2.

Claim 22(Original): The method according to claim 21 wherein R^3 is an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$, wherein $C(R)_3$ is C_6H_5 aromatic; R^1 and R^2 are each hydrogen; j is 1; and B is a bridging group of the formula: -$(CR^1R^2)_k$-C(=O)-Z-, wherein k is 5; and Z is O.

Claim 23(Original): The method according to claim 21 wherein R^3 is an endosome membrane disruption promoting component of the formula -B-(R^4)R, wherein B is a bridging group of the formula: -$(CR^1R^2)_k$-C(=O)-Z-; R is absent, R^1 and R^2 are each hydrogen; k is 5, Z is absent; and R^4 is a fusogenic peptide.

Claim 24(Original): The method according to claim 21 wherein R^3 is an endosome membrane disruption promoting component of the formula -B-(R^4)R, wherein B is a bridging group of the formula: -$(CR^1R^2)_{j=}$-X-; R is absent, R^1 and R^2 are each hydrogen; j= is 5, X is N(R); and R^4 is a cholic acid derivative wherein R^6 is OH, R^7 is C_3 alkylcarbonyl and R^8 is C_1 alkyl.

Claim 25(Original): The method according to claim 21 wherein R^3 is an endosome membrane disruption promoting component of the formula -B-(R^5)R, wherein R is absent and B is a bridging group of the formula: -(CR^1R^2)$_k$-N(R)-C(=O)-Z- in which R, R^1 and R^2 are each hydrogen; k is 5, Z is absent; and R^5 is α-3'-propionyl thiomannoside.

Claim 26(Original): The method according to claim 21 wherein R^3 is an endosome membrane disruption promoting component of the formula -B-(CR^1R^2)$_j$-C(R)$_3$, wherein C(R)$_3$ is C_6H_5 aromatic; R^1 and R^2 are each hydrogen; j is 1 and B is a bridging group of the formula: -(CR^1R^2)$_k$-N(R)-C(=O)-Z-; k is 5, N(R) is NH and Z is O.

Claim 27(Original): The method according to claim 8, wherein the cationic polyamine has the formula NH(R^{30})-(CH_2)$_3$-N(R^3)-(CH_2)$_4$-N(R^3)-(CH_2)$_3$-NH(R^{30})
wherein:

R^{30} is hydrogen or NH(R^{30}) is guanidino;

at least one R^3 is an endosome membrane disruption promoting component of the formula -B-(CR^1R^2)$_j$-C(R)$_3$.

Claim 28(Original): The method according to claim 27 wherein:

R^{30} is hydrogen; and

each R^3 is an endosome membrane disruption promoting component of the formula -B-(CR^1R^2)$_j$-C(R)$_3$,

wherein C(R)$_3$ is C_6H_5 aromatic; R^1 and R^2 are each hydrogen; j is 1; and B is a bridging group of the formula: -(CR^1R^2)$_k$-N(R)-C(=O)-Z-;

where k is 5; N(R) is NH; and Z is O.

Claim 29(Original): The method according to claim 27 wherein:

R^{30} is hydrogen; and

each R^3 is an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$,

wherein B is absent, R, R^1 and R^2 are each hydrogen; and j is 7.

Claim 30(Original): The method according to claim 27 wherein:

$NH(R^{30})$ is guanidino; and

each R^3 is an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$,

wherein B is absent, R, R^1 and R^2 are each hydrogen; and j is 7.

Claim 31(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

one R^3 is hydrogen; and

one R^3 is an endosome membrane disruption promoting component of the formula -B-(R^4)-R,

wherein R is absent and B is a bridging group of the formula:

-$(CR^1R^2)_{j=}$-X-, in which R, R^1 and R^2 are each hydrogen; j= is 5; and X is N(R) and

where R^4 is a type (iii) cholesteryl derivative of formula (3):

R^{6a} is O-C(=O)- and a point of attachment of cholesteryl derivative;

R^{7a} is C_5 alkyl; and

R^{8a} is C_1 alkyl.

Claim 32(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

each R^3 is an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$,

wherein B is a bridging group of the formula: -$(CR^1R^2)_k$-C(=O)-Z-; R^1 and R^2 are each hydrogen; j is 0, k is 11; Z is N(R) where R is C_1 alkyl and $C(R)_3$ is CH_3.

Claim 33(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

each R^3 is an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$;

wherein B is a bridging group of the formula: -$(CR^1R^2)_k$-C(=O)-Z-; R^1 and R^2 are each hydrogen; j is 1, k is 11; Z is O and $C(R)_3$ is C_6H_5 aromatic.

Claim 34(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

each R^3 is an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$;

wherein B is a bridging group of the formula: -$(CR^1R^2)_k$-C(=O)-Z-; R^1 and R^2 are each hydrogen; j is 0, k is 11; Z is OH and $C(R)_3$ is absent.

Claim 35(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

one R^3 is hydrogen; and

one R^3 is an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$;

wherein B is a bridging group of the formula: $-(CR^1R^2)_k-C(=O)-Z-$; R^1 and R^2 are each hydrogen; j is 1, k is 11; Z is O and $C(R)_3$ is C_6H_5 aromatic.

Claim 36(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

one R^3 is hydrogen; and

one R^3 is an endosome membrane disruption promoting component of the formula $-B-(CR^1R^2)_j-C(R)_3$;

wherein B is a bridging group of the formula: $-(CR^1R^2)_k-C(=O)-Z-$; R^1 and R^2 are each hydrogen; j is 0, k is 11; Z is OH and $C(R)_3$ is absent.

Claim 37(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

each R^3 is an endosome membrane disruption promoting component of the formula $-B-(R^5)R$;

wherein R is absent and B is a bridging group of the formula: $-(CR^1R^2)_k-N(R)-C(=O)-Z-$, in which R, R^1 and R^2 are each hydrogen; k is 5; Z is absent and

R^5 is α-3'-propionyl thiomannoside.

Claim 38(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

one R^3 is hydrogen; and

one R^3 is an endosome membrane disruption promoting component of the formula $-B-(R^5)R$;

wherein R is absent and B is a bridging group of the formula: $-(CR^1R^2)_k-N(R)-\{-(C=O)-CH_2-O-[-(CH_2)_2-O-]_l-(CH_2)_k-N(R)\}_p-C(=O)-Z-$ in which R, R^1 and R^2 are each hydrogen; k is 5; l is 5; p is 1; Z is absent; and

R^5 is α-3'-propionyl thiomannoside.

Claim 39(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

one R^3 is hydrogen; and

one R^3 is an endosome membrane disruption promoting component of the formula $-B-(R^5)R$;

wherein R is absent and B is a bridging group of the formula: $-(CR^1R^2)_k-N(R)-\{-(C=O)-CH_2-O-[-(CH_2)_2-O-]_l-(CH_2)_k-N(R)\}_p-C(=O)-Z-$ in which R, R^1 and R^2 are each hydrogen; k is 5; l is 20; p is 1; Z is absent; and

R^5 is α-3'-propionyl thiomannoside.

Claim 40(Original): The method according to claim 27 wherein:

R^{30} is hydrogen;

one R^3 is hydrogen; and

one R^3 is an endosome membrane disruption promoting component of the formula $-B-(R^5)R$;

wherein R is absent and B is a bridging group of the formula: $-(CR^1R^2)_k-N(R)-\{-(C=O)-CH_2-O-[-(CH_2)_2-O-]_l-(CH_2)_k-N(R)\}_p-C(=O)-Z-$ in which R, R^1 and R^2 are each hydrogen; k is 5; l is 5; p is 1; Z is absent; and

R^5 is N^2, N^6-bis(β-3'-propionyl galactosyl-β1-4-thioglucoside)lysyl-N^6-(β-3'-propionyl galactosyl-β1-4-thioglucoside)lysine.

Claim 41(Original): The method according to claim 8, wherein said transfer moiety comprises more than one cationic polyamine component.

Claim 42(Original): The method according to claim 8, wherein a first cationic polyamine component comprises an endosome membrane disruption promoting component and a second cationic polyamine component comprises a receptor specific binding component.

Claim 43(Original): The method according to claim 42, wherein the first cationic polyamine component has an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$, wherein $C(R)_3$ is absent, R^1 and R^2 are each hydrogen; j is 0 and B is a bridging group selected from the group consisting of (i), (ii), (iii) and (iv).

Claim 44(Original): The method according to claim 42, wherein the first cationic polyamine component has an endosome membrane disruption promoting component of the formula -B-$(CR^1R^2)_j$-$C(R)_3$, wherein $C(R)_3$ is absent, R^1 and R^2 are each hydrogen; j is 0 and B is a bridging group of the formula: -$(CR^1R^2)_k$-C(=O)-Z-; k is 11 and Z is OH.

Claim 45(Original): The method according to claim 42, wherein the first cationic polyamine component has an endosome membrane disruption promoting component of the formula -B-(R^4)R, wherein R^4 is a cholesteryl derivative.

Claim 46(Original): The method according to claim 42, wherein the first cationic polyamine component has an endosome membrane disruption promoting component of the formula -B-(R^4)R, wherein R is a absent and B is a bridging group of the formula: -$(CR^1R^2)_{j=}$-X-, in which R, R^1 and R^2 are each hydrogen; j= is 5; and X is N(R) and where R^4 is a type (iii) cholesteryl derivative of formula (3): R^{6a} is O-C(=O)- and a point of attachment of cholesteryl derivative; R^{7a} is C_5 alkyl; and R^{8a} is C_1 alkyl.

Claim 47(Original): The method according to claim 42, wherein the receptor specific binding component of said second cationic polyamine component is selected from the group consisting of:

β-3= propionyl galactosyl-β1-4-thioglucoside;

N^2, N^6-bis(β-3'-propionyl galactosyl-β1-4-thioglucoside)lysine;

N^2, N^6-bis(β-3'-propionyl galactosyl-β1-4-thioglucoside)lysyl-N^6-(β-3'-propionyl galactosyl-β1-4-thioglucoside)lysine;

α-3'-propionyl thiomannoside; and

α-3'-propionyl thiomannoside-6-phosphate.

Claim 48(Original): A method for delivering a nucleic acid molecule to a targeted population of cells of an individual, said method comprising the step of delivering to the individual a multifunctional molecular complex comprising:

A) a nucleic acid molecule; and

B) a transfer moiety comprising one or more cationic polyamine components, wherein each cationic polyamine is non-covalently bound to said nucleic acid molecule and each independently comprises a cationic polyamine of the formula (1):

$$NR(R^3)\text{-}[\text{-}(CR^1R^2)_m\text{-}N(R^3)\text{-}]_n\text{-}(CR^1R^2)_m\text{-}NR(R^3) \quad (1)$$

wherein:

R, R^1 and R^2 are each independently selected from the group consisting of hydrogen and $C_{1\text{-}6}$ alkyl;

m in each occurrence is independently selected from the integers 2 through 5 inclusive;

n is selected from the integers 1 through 10 inclusive;

R^3 is independently selected from the group consisting of hydrogen; C_{1-6} alkyl, and an endosome membrane disruption promoting component, or $NR(R^3)$ is guanidino;

wherein said transfer moiety comprises at least one endosome membrane disruption promoting component attached to at least one nitrogen atom of at least one of said cationic polyamine components;

wherein said transfer moiety comprises at least one receptor specific binding component attached either (i) to a further nitrogen atom of at least one of said cationic polyamine components to which said one or more endosome membrane disruption promoting components is attached, or (ii) to a nitrogen atom of at least one further polyamine component which does not have attached thereto any endosome membrane disruption promoting component,

wherein said receptor specific binding component which is a ligand for natural receptors of said target cells.

Claim 49 (New): A method according to Claim 2 wherein the peptide, protein or nucleic acid molecule is a therapeutic agent.

Claim 50 (New): A method for the transfer of a nucleic acid composition to cells, comprising the step of introducing a multifunctional molecular complex into cells, wherein said multifunctional molecular complex comprises:

(a) a nucleic acid molecule; and

(b) a transfer moiety comprising:

(i) one or more cationic polyamine components, wherein each cationic polyamine is non-covalently bound to a nucleic acid composition and comprises from three to twelve nitrogen atoms; and

(ii) one or more endosome membrane disruption promoting components independently selected from the group consisting of:

(a) at least one lipophilic long chain alkyl group attached to a nitrogen atom of said polyamine,

(b) a fusogenic peptide attached to a nitrogen atom of said polyamine through a short alkyl bridging group having a terminal carboxyl, amino, hydroxyl or sulfhydryl group, and

(c) a cholic acid or cholesteryl or a derivative thereof attached to a nitrogen atom of said polyamine through a short alkyl bridging group having a terminal carboxyl, amino, hydroxyl or sulfhydryl group,

wherein said multifunctional molecular complex transfers said nucleic acid composition to said cells.

Claim 51 (New): The method according to claim 50, wherein said transfer moiety further comprises at least one receptor specific binding component which is a ligand for a receptor on a target cell.

Claim 52 (New): The method according to claim 50, wherein the receptor specific binding component is attached through a bridging group to either (i) to a further nitrogen atom of at least one of said cationic polyamine components to which said one or more endosome membrane disruption promoting components is attached, or (ii) to a nitrogen atom of at least one further polyamine component which does not have attached thereto any endosome membrane disruption promoting component.

(IX) Evidence Appendix

Attached hereto is a copy of the Rule 1.132 Declaration filed by Applicant on March 5, 2004.

AHP1AUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	) Group Art Unit: 1632
Raymond H. Boutin	) Examiner: D. Crouch
Appln No. 08/809,397	)
Filed: March 21, 1997	) November 20, 1998
For: MULTIFUNCTIONAL MOLECULAR COMPLEXES FOR GENE TRANSFER TO CELLS	)

Asst. Commissioner for Patents
Washington, DC 20231

DECLARATION PURSUANT TO 37 CFR 1.132

Sir:

I, Julia Schauer, residing at 140 Swinehart Road, Coatesville, Pennsylvania 19320, a citizen of the United States of America, do declare and state that:

1. I am employed by assignee of the above-identified patent application and I am a member of the project team responsible for the subject matter of this application.

CERTIFICATE UNDER 37 CFR 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail on the date indicated below in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231.

Signature _____

Date 1-12-99

2. My educational background includes a bachelor of science in Chemistry and additional pre-medical course work. I have had fourteen years of experience in the field of drug design and vaccine development and I am currently so employed. A copy of my résumé is attached.

3. I have read and understood the specification of this application.

4. I understand that the data provided herein is being presented to provide additional evidence to support the utility of the pharmaceutical compositions of the invention. The experiments described herein were performed or coordinated by me.

5. The experiment described in Paragraphs 6 and 7 below were performed to determine the ability of the multifunctional molecular complexes of the invention to transfer nucleic acids. The data demonstrates that several different multifunctional molecular complexes according to claim 1 enhance expression of plasmid DNA gene products in cultured cells as compared to control cells which were treated with DNA alone.

6. Four exemplary multifunctional molecular complexes according to claim 1, containing DNA and a transfer moiety selected from bis-octyl-bis-guanidino spermine, cholesteryl spermidine, benzyldodecyl spermidine, or cholesterol spermine were prepared as described in the specification. Briefly, plasmid DNA (2 μg) coding for the luciferase enzyme was mixed with the transfer moieties identified above at various ratios and allowed to form multifunctional molecular complexes according to claim 1. These complexes, as well as naked DNA, were applied to human rhabdomyosarcoma (RD) cells at approximately 80% confluency. After 4 hours, the cells were washed, given fresh media and incubated for up to 48 hours. The cells were lysed, and the gene product was measured by a standard luciferase assay.

7. The results are illustrated in Exhibits 1 through 4, attached. As is readily apparent from these Exhibits, transfection of human RD cells by DNA complexed in each of the exemplary transfer moieties of the invention was enhanced, as compared to transfection of the cells by DNA alone.

8. Thus, the experiments presented in Paragraphs 6 and 7 demonstrate that a variety of different multifunctional molecular complexes of the invention enhance the expression of plasmid DNA gene product in cultured cells over cells treated with DNA alone.

9. In a series of experiments similar to those described in Paragraph 6, multifunctional molecular complexes of the invention containing DNA complexed with cholesteryl spermine or cholesteryl spermidine, have been demonstrated to mediate transfection of a variety of cell types *in vitro*. Although performance varied by cell type, use of the multifunctional molecular complexes of the invention resulted in the transfer of complexed DNA in cell types including, among others, human rhabdomyosarcoma (RD), human hepatocellular carcinoma (HUH 7), human hepatocytes (FOCUS), human hepatoblastoma (HEP 2), mouse embryo (NIH/3T2), human colon carcinoma (LS 180), SV40 transformed monkey kidney (COS-1), human osteosarcoma (U-2 OS), adenovirus-transformed human kidney cells (293), human breast cancer (MCF7), human neuronal blastoma (SY5Y), and primary human hepatocytes. These experiments demonstrated the utility of the multifunctional molecular complexes of the invention in mediating transfer of DNA in a wide variety of cell types.

10. The experiment described in Paragraphs 11 - 15 demonstrate the ability of the pharmaceutical compositions of the invention to induce immune responses *in vivo*.

11. A multifunctional molecular complex of the invention, containing 50 μg plasmid DNA coding for the gD protein of herpes simplex virus type 2 (HSV-2) and transfer moiety cholesteryl spermine (CSm), was prepared as described in the specification. An equivalent amount of plasmid DNA (50 μg) was (a) uncomplexed and thus administered as naked DNA or (b) complexed with 0.25% bupivacaine. To form a pharmaceutical composition of the invention, the multifunctional molecular complex of the invention was formulated with 20% v/v polyethylene glycol 300 USP/NF in water. The naked DNA and the DNA complexed with bupivacaine were similarly formulated. The pharmaceutical composition of the invention, the naked DNA, and the DNA complexed with bupivacaine were each administered intramuscularly (im) as a single dose to mice. The cellular and humoral immune responses against gD were measured after 30 days as described in Paragraphs 12 and 13.

12. Cellular responses were measured by carrying out a lympho-proliferation assay on spleen cells of mice that received the compositions described in Paragraph 11. For the pharmaceutical compositions containing the multifunctional molecular complex of the invention, the charge ratios of transfer moiety to DNA were varied between 0.7:1, 1.0:1, and 1.5:1. The spleen cells were cultured with or without antigen, and the counts were recorded as delta counts per million [DCPM]. The results are illustrated in Exhibit 5, attached.

13. Humoral responses were measured by carrying out a standard ELISA assay on the serum samples obtained from mice that received the compositions described in Paragraph 11. For the pharmaceutical compositions containing the multifunctional molecular complex of the invention, the charge ratios of transfer moiety to DNA were varied between 0.7:1, 1.0:1, and 1.5:1. The quantitative measure of antigen specific antibody is expressed as optical density at 450 nm (OD_{450}). These results are illustrated in Exhibit 6, attached.

14. Following one intramuscular injection of a pharmaceutical composition of the invention, both cellular and humoral immune responses were measured. This experiment shows that mice that received 0.7:1 ratio of CSm:DNA had higher cellular (Exhibit 5) and humoral (Exhibit 6) responses compared to animals receiving naked DNA, DNA complexed with bupivacaine or DNA complexed with the transfer moieties at ratios of 1.0:1 and 1.5:1. Because this was a first-round experiment, the results are non-optimal, yet the ability of the pharmaceutical compositions of the invention to induce an immune response is clearly demonstrated. Further, the ability of the pharmaceutical compositions of the invention to enhance the immune response over naked DNA is evident.

15. The *in vivo* experimental data presented in Paragraphs 10 to 14 correlates with the experimental evidence described in Paragraph 9, which demonstrates the ability of the multifunctional molecular complexes of the invention to mediate transfection of a variety of cell types. Thus, the data provided herein supports the ability of the pharmaceutical compositions of the invention to successfully transfer DNA to host cells and that the DNA is expressed in a manner which elicits an immune response.

16. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 11-24-98 By: Julia Schauer
Julia Schauer

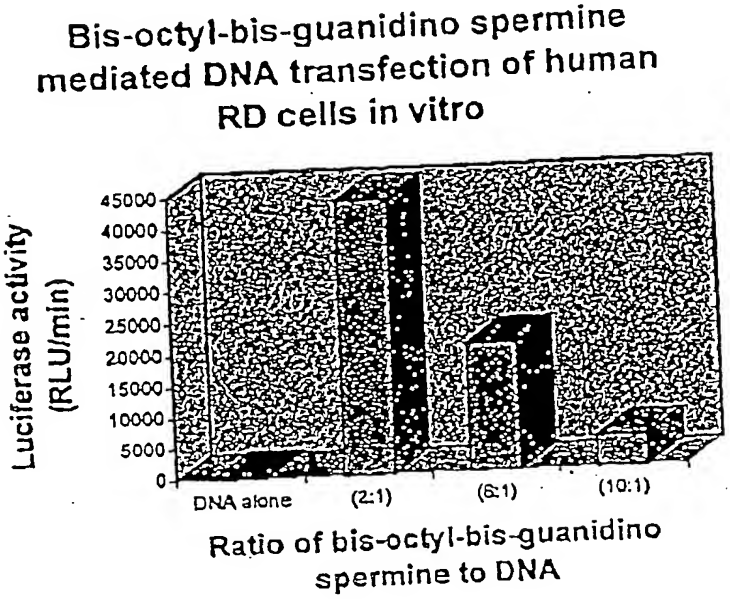


Exhibit 1
Rule 132 Declaration
08/809,397

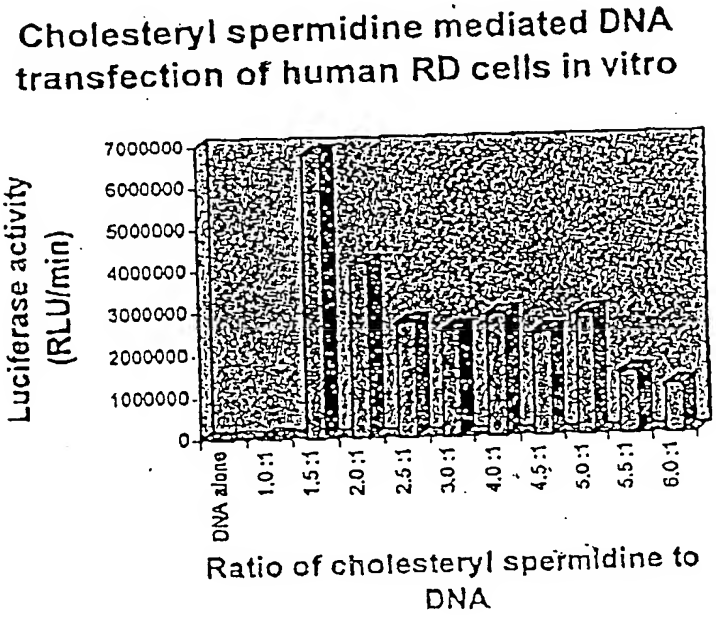


Exhibit 2
Rule 132 Declaration
08/809,397

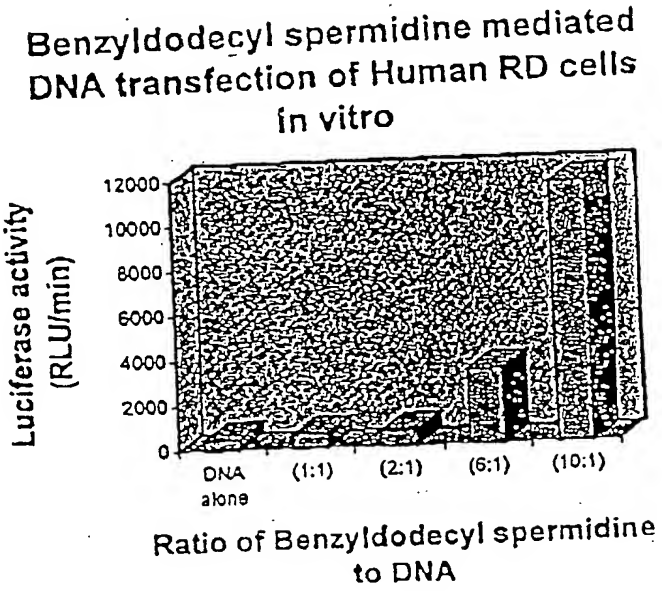


Exhibit 3
Rule 132 Declaration
08/809,397

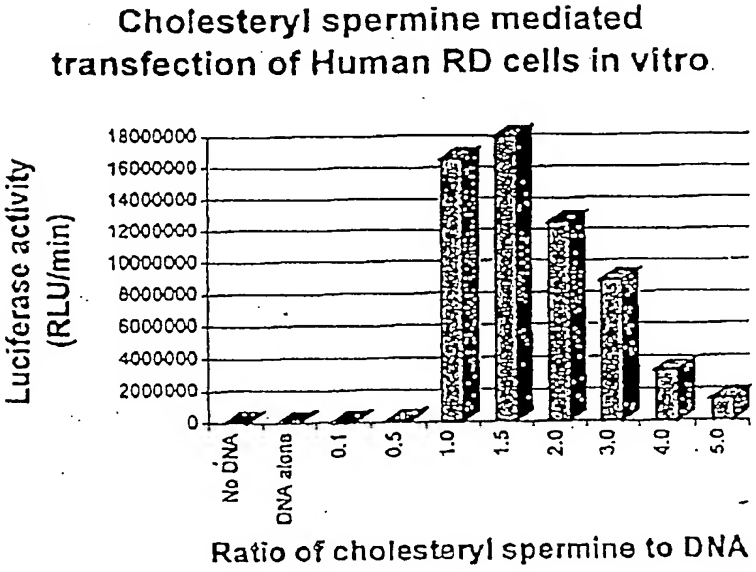


Exhibit 4
Rule 132 Declaration
08/809,397

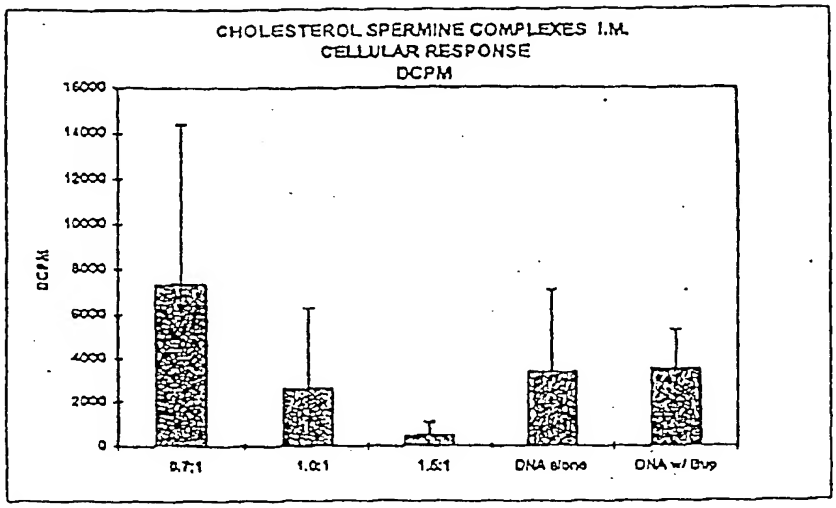


Exhibit 5
Rule 132 Declaration
08/809,397

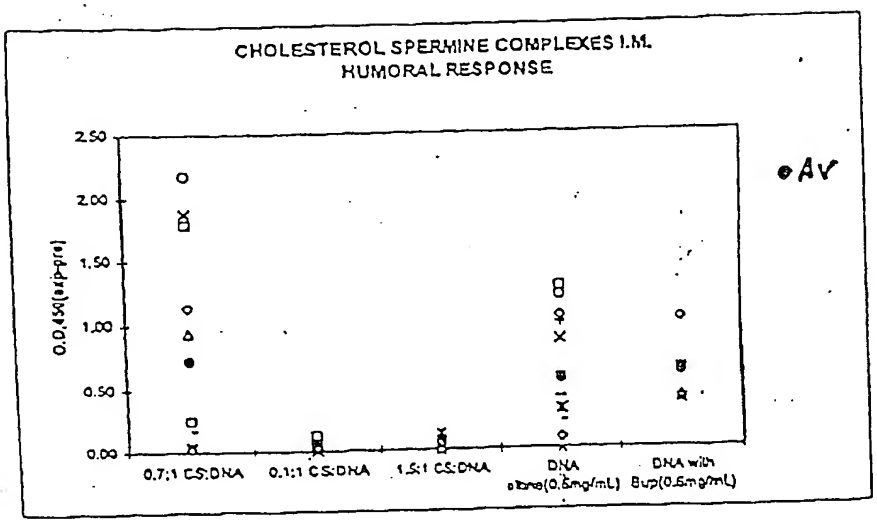


Exhibit 6
Rule 132 Declaration
08/809,397

JULIA SCHAUER
140 Swinehart Road
Coatesville, Pennsylvania 19320
(610) 383-6358

OBJECTIVE: Pharmaceutical or biotechnology research position which utilizes my pre-medical / chemistry education and extensive experience in organic synthesis, analytical chemistry, protein chemistry, radiochemistry, molecular and cellular biology, and formulations development.

QUALIFIED BY:
* Fourteen years of drug design and vaccine development research experience with increasing responsibilities in project design and management.
* Bachelor of Science in Chemistry plus additional pre-medical course work. (Honors graduate).
* Excellent oral and written communication skills.

"Julia has demonstrated herself to be an excellent communicator and to have the ability to effectively supervise situations and personnel in a matrix structure. We need more employees like this."

Dr. Richard Carrano, Vice President, Technology Devmt. and Regulatory Affairs
1994 Performance Review Excerpts, Apollon, Inc.

EXPERIENCE: American Home Products, DNA Vaccines Division (formerly Apollon, Inc.), Malvern, PA

1996-present ***Associate Research Scientist, Molecular and Cellular Biology and Chemistry R&D***
1993-1996 ***Senior Research Associate, Formulations and Chemistry R&D***
1992-1993 ***Research Associate, Chemistry R&D***

My principal duties involved invention and development of novel strategies to deliver gene products to cells. I was involved in all stages of reducing these concepts to practice and testing the resulting product candidates. My areas of technical expertise include:

- *Invention of novel non-viral delivery vehicles for transmembrane passage of DNA.*
  Invented several classes of compounds designed to bind plasmid DNA and exploit both receptor specific and non-specific pathways for uptake into cells. Actively studied the current developments in the field, and lead group discussions at company and team meetings.

- *Design and execution of multistep synthesis of complex organic molecules.*
  Conducted the total synthesis of a variety of molecules including carbohydrates, steroids, peptides, polyamines, and various biomolecules. Thoroughly characterized products by FT NMR (300 MHz), Elemental Analysis, FAB Mass Spectroscopy, HPLC and TLC. Supervised scale up of material for testing, and trained a junior level chemist.

- *Preparation and purification of modified proteins, antibodies and antibody fragments.*
  As the point person in a collaboration with the Molecular Hepatology Dept. at Harvard Medical School, I prepared conjugated IgG antibodies utilizing my own bifunctional ligands. The resulting antibody conjugates were complexed with DNA and tested for receptor targeted cellular uptake in vitro.

- *Development of radioactive assays.*
  Trained with over ten years of experience in handling radioactivity. Prepared and utilized radioactive probes for elucidating DNA complexation mechanisms.

- *Optimization of cultured cell transfection methods for in vitro studies.*
  Conducted cell culture experiments utilizing sterile technique to screen product candidates for efficacy in vitro. Independently optimized the assay and identified the lead compound.

- *Development of formulations methods for evaluation of product stability and efficacy in vivo.*
  Conducted stability studies and physico-chemical characterization of DNA complexes including dynamic light scattering, zeta potential determination, flow cytometric sizing and light microscopy.

1987-1992 Centocor, Inc Malvern, PA

1991-1992 *Research Associate, Radiochemistry R&D*
1987-1991 *Senior Research Assistant, Radiochemistry R&D*

- Designed, prepared and tested radioactive imaging agents for cancer and heart disease.
- Prepared and purified modified monoclonal antibody entities as new diagnostic agents.
- Supervised scale up preparation of a new product for use in several human clinical trials.

1984-1986 Johnson Matthey, Inc., West Chester, PA

Junior Chemist, Drug Delivery Group, Chemistry R&D

- Conducted organic synthesis of molecules for attachment to platinum as new chemotherapeutic drugs.

EDUCATION: ***West Chester University, West Chester, PA***
Bachelor of Science in Chemistry, 1984.
Cumulative GPA 3.8/4.0, Summa Cum Laude Honors Graduate.

PUBLICATIONS:

Pachuk CJ*, Satishchandran C*, Bayer ME, Samuel M, Zurawski DV, Troutman RD, **Schauer** JI and Ciccarelli RB. *Bupivacaine forms liposomal complexes with DNA.* (in preparation).

L. Mohr, **J.I. Schauer**, R.H. Boutin, D. Moradpour, J.R. Wands. *Targeted gene transfer to hepatocellular carcinoma cells using a novel monoclonal antibody based gene delivery system.* (Manuscript accepted by Hepatology for publication in late 1998).

L. Mohr, **J.I. Schauer**, D. Moradpour, R.H. Boutin, R.B. Ciccarelli, J.R. Wands, and V.R. Zurawski Jr. *Efficient Gene Delivery with Spermidine- and Spermine- Compounds.* Abstract of Papers presented at 1996 Meeting on Gene Therapy, Sept 25-29, 1996, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Darius Moradpour, **Julia I. Schauer**, Vincent R. Zurawski, Jr., Jack R. Wands, and Raymond H. Boutin. *Efficient gene transfer into mammalian cells with cholesteryl-spermidine.* Biochemical and Biophysical Research Communications. 221,82-88 (1996).

Mark A. Nedelman, David J. Shealy, Raymond Boutin, Eva Brunt, **Julia I. Seasholtz** (former name), I. Elaine Allen, John E. McCartney, Frederick D. Warren, Herman Oppermann, Roy H.L. Pang, Harvey J. Berger and Harlan F. Weisman. *Rapid Infarct Imaging with a Technetium-99m-Labeled Antimyosin Recombinant Single-Chain Fv: Evaluation in a Canine Model of Acute Myocardial Infarction.* The Journal of Nuclear Medicine, Vol. 34, No.2, 234-241. (1993).

(X) Related Proceedings Appendix

None applicable.